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A molecular (PCR) survey on abortions caused by *Campylobacter spp.* in sheep flocks located on the suburb of Tabriz

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Abstract

Campylobacteriosis is an important infectious disease of animals and humans caused by the pathogenic *Campylobacter* species. A total number of 132 aborted sheep fetuses and related placentas were admitted to the large animal clinic at the University of Tabriz, from October 2010 to March 2011. Tissue samples were collected from several fetal organs including liver, brain, kidney, lung, spleen, heart, stomach fluid and placenta, then separately pulverized under liquid nitrogen and finally stored at -20°C until DNA extraction. Of 132 submissions (fetuses and placentas), 12 (9.09%) and 2(1.51%) samples were diagnosed positive to the *Campylobacter fetus* subsp. *fetus* and *Campylobacter jejuni* by the PCR protocol, respectively. No samples were positive for *Campylobacter coli*.

Keywords: Campylobacteriosis, abortion, sheep, PCR

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Introduction

Abortion in sheep may be induced by numerous factors, whether of infective or non-infective nature. Among the infectious abortions, campylobacteriosis is more important in many countries (Salihu *et al*., 2009; Uaboi-Egbenni *et al*., 2010; Sippy *et al*., 2012). *Campylobacter* was first isolated from aborted sheep fetuses in 1909 and given its current name in 1963. These organisms cause two major groups of disease: foetal infections in cattle and sheep and acute enterocolitis in humans (Sudworth, 2001). This disease is caused by *Campylobacter fetus subsp. fetus* or *Campylobacter jejuni*. Both organisms can cause epidemics of abortion characterized by gross lesions in the placenta and/or foetal tissues (Means, 2007; Peel and Mason, 1993). In Denmark and New Zealand it has been reported that more than 60% of sheep abortions are associated with *C. fetus subsp. fetus* and *C. jeuni* infections. (Agerholm *et al*., 2006; Mannering *et al.*, 2006).

Thermophilic campylobacters such as *C. coli* are also known as causal agents of abortions in sheep (Butzler, 2004). Moreover, it causes acute gastroenteritis in human and enteritis in animals (Tangvatcharin *et al*. 2005). Infected and often symptomless animals excrete these organisms in feces. *C. jejuni* is mainly from a wildlife source and *C.fetus fetus* from carrier sheep. The rout of infection in sheep is mainly by oral (Noakes *et al*., 2001). A better understanding of the epidemiology of *Campylobacter* infection is important in prevention and control of sheep abortion. This study was conducted to establish the presence and detection of *C. fetus subsp*. *fetus*,_C. *jeuni* and *C. coli*_in aborted sheep fetuses in Tabriz suburb.

Materials and methods

Samples

A total number of 132 aborted fetuses and related placentas were admitted to the large animal clinic at the University of Tabriz, from October 2010 to March 2011.Tissue samples were collected from several fetal organs including liver, kidney, lung, brain, spleen, heart, stomach fluid and placenta, then separately pulverized under liquid nitrogen and finally stored at -20°C until DNA extraction.

DNA Extraction

DNA extraction from frozen tissues samples was performed using a commercial kit (Accuprep Genomic DNA Extraction Kit, Bioneer, S. Korea) following the manufacturer's instructions. Briefly, 100 μL of thawed homogenates of fetal tissues were mixed with 600 μL of Nuclei Lysis Solution and homogenized for 10 seconds. Samples were incubated at 65°C for 30 min, followed by the addition of 17.5 μL proteinase K (20 mg mL-1) and incubation at 60°C for 3 h, vortexing every 30 min. Three microliters of RNase A (4 mg mL-1) were added, the samples were mixed and incubated at 37°C for 30 min. After cooling, 200 μL of protein precipitation solution were added, followed by vortexing and centrifugation at 13,000 g for 4 min. The supernatant was transferred to a new microtube with 600 μL of isopropanol, mixed, and centrifuged at 13,000 g for 3 min. The supernatant was discarded and the pellet was washed with 600 μL of 70% ethanol, followed by a final centrifugation at 13,000 g for 3 min. Each pellet was dissolved in 100 μL of DNA Rehydration Solution by incubating at 65°Cfor 1 h. DNA quality was assessed by spectrophotometry and samples that had DNA concentration lower than 100ng μL^{-1} were excluded from further analysis.

PCR

PCR was used for detection of pathogenic *Campylobacter spp.* PCR reactions were performed using 13 μL of a commercial PCR mix (Accupower PCR preMix, Bioneer, S. Korea), 0.75 μL of a 25 μM solution of each primer (Table 1), and 1 μL of DNA (100 to 500 ng per reaction). Parameters used were

initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 58°C for 1 min, extension at 72°C for 1 min and a final extension of 72°C for 7 min. Positive controls (Genekam Co., Germany) from *Campylobacter Genus* and negative controls (in which DNA template was replaced by PCR-grade water) were included in all reactions. PCR products were resolved by electrophoresis in a 1.5% agarose gel stained with ethidium bromide.

Results

Of 132 submissions (fetuses and placentas), 12 (9.09%) and 2(1.51%) samples were diagnosed positive to the *Campylobacter fetus subsp. fetus* and *Campylobacter jejuni* by the PCR protocol, respectively. No samples were positive for *Campylobacter coli* (Table 2, Fig1&Fig2).

Discussion

Pregnancy losses caused by a variety of infectious agents produce a severe economic impact on the profitability of the sheep industry worldwide (Campero *et al*., 2005). Campylobacteriosis is the important cause of abortion in the sheep in many of countries including Iran (Tadjbakhsh, *et al*., 2000; Firouzi, R. 2006; Ekin *et al*., 2006; Sadeghi *et al*., 2008; Salihu *et al*., 2009; Uaboi-Egbenni, *et al*., 2010). This disease is a highly contagious and economically significant disease in sheep and is most often caused by the bacteria *Campylobacter fetus* subsp. *fetus* and *C. jejuni* which cause abortion in sheep (Hedstromr *et al*., 1987). Infection occurs through ingestion of the organism. Most abortions occur in the last month of pregnancy. Unlike the cow, sexual transmission and infertility are not features of *campylobacter* infections in sheep. In humans, *Campylobacter jejuni* is recognized to be a common cause of acute diarrhea, and is associated with abortion and neonatal sepsis. (Simor *et al*., 1986) The disease is very contagious and spreads rapidly among the remaining ewes unless very strict hygiene is practiced. The fetus, placenta, birth fluids, vaginal discharge, and feces from the ewe are all sources of infection. If the water or feeding areas become contaminated with these materials, the abortion rate can be very high.

Table 1. Primer sequences for *Campylobacter Coli & Fetus & jejuni* [Hum *et al*. (1997), Persson and Olsen (2005)]

Bacterial name	Primers sequence	PCR product Molecular weight(bp)
Campylobacter Genus F	5-GGA TGA CAC TTT TCG GAG C-3	816
Campylobacter Genus R	5-CAT TGT AGC ACG TGT GTC-3	816
Campylobacter Coli F	5-GGT ATG ATT TCT ACA AAG CGA G-3	502
Campylobacter Coli R	5-ATA AAA GAC TAT CGT CGC GTG-3	502
Campylobacter Fetus F	5-GGA AGC CGC AGC TGC TAA GAT-3	359
Campylobacter Fetus R	5-AGC CAG TAA CGC ATA TTA TAG TAG-3	359
Campylobacter Jejuni F	5-CAA ATA AAG TTA GAG GTA GAA TGT-3	161
Campylobacter Jejuni R	5-CCA TAA GCA CTA GCT AGC TGA T-3	161

Table 2. Results of PCR tests for diagnosis of *Campylobacter spp.* in the aborted fetal tissues

Campylobacter spp.	Positive	Negative
C. fetus subsp. fetus	12(9.09%)	120(90.91)
C. jejuni	2(1.5%)	130(98.5%)
C. coli	-	132(100%)

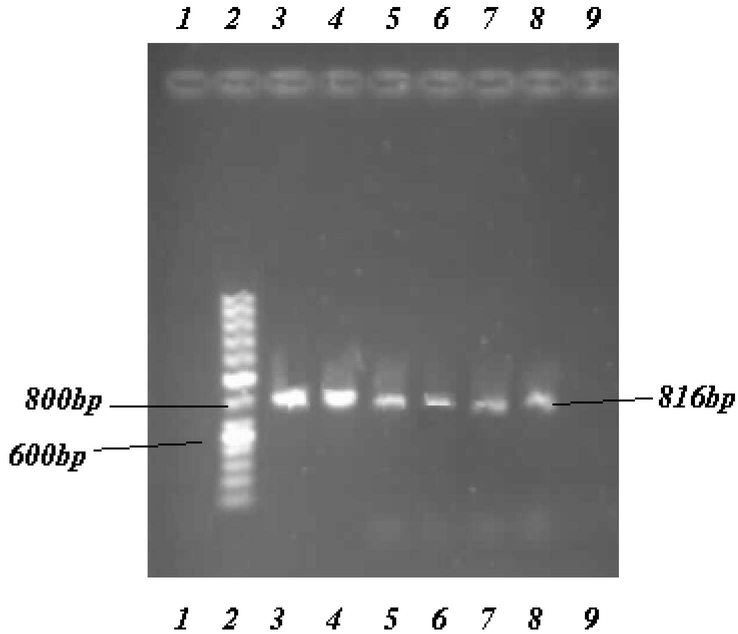


Figure 1. Representative results of PCR amplification of genomic DNA of *Campylobacter Genus* in fetal tissues. Lane 1: Non template control (NTC), Lanes 2: 100 bp molecular weight marker (Bioneer, S. Korea), 3: positive control (Genekam Co., Germany), 4-8: positive samples from aborted fetuses, negative samples from aborted fetuses, and 9: Negative control

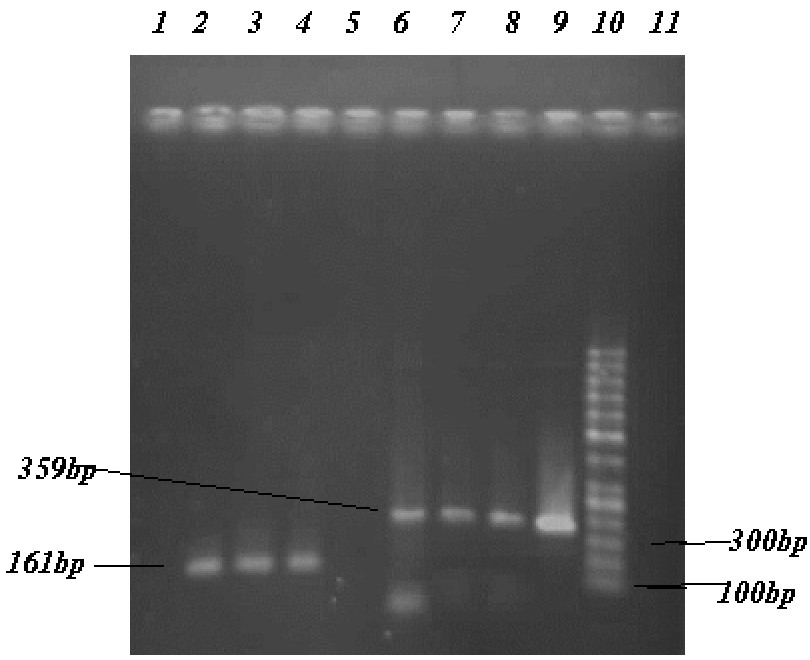


Figure 2. Representative results of PCR amplification of genomic DNA of *Campylobacter spp.* in fetal tissues. Lane 1: Non template control (NTC), Lanes 2: positive control for *C. jejuni* (Genekam Co., Germany), 3&4: positive samples for *C. jejuni* , 5: empty, 6: positive control for *C.fetus* subsp. *fetus*, 7-9: positive samples for *C. fetus* subsp. *fetus*, 10: 100 bp molecular weight marker (Bioneer, S. Korea) and11: negative control

Our results indicated that campylobacteriosis is an important cause of abortion in sheep and *Campylobacter fetus subsp. fetus* has the main role in ewe's abortion in Tabriz region. These results in accordance with results obtained by Tadjbakh *et al*.(2000) and Fenwick *et al*.(2000) that identified *C. fetus subsp. fetus* as the causal agent of the ewes abortions in Iran(Tehran & Esfahan) and New Zealand, respectively (Tadjbakhsh , *et al*., 2000; Fenwick *et al*.2000).

On the other hand, our results are in contrast with the results obtained by Shahrokhabadi *et al*. (2013) Ekin *et al*. (2006) and Salihu *et al*. (2009) that emphasized on *C. jejuni* and *C. coli* as the most important campylobacter species in sheep diseases and abortion in Zahedan (Iran), Turkey, and Nigeria, respectively.

In conclusion Campylobacteriosis is a very important disease in sheep abortion in Tabriz area and responsible for 10.6% of sheep abortions in this region. Among the *Campylobacter* species, *C.fetus subs. fetus* is the most important pathogenic *campylobacter* in our region and responsible for 9.09% of ewe's abortions, whereas *C. jejuni* and *C. Coli* have the minor roles in this case (1.5% of all of abortions). Therefore, vaccination of ewes by a formalin-killed adjuvant vaccine incorporating of *C.fetus subs. fetus* before the breeding season could be a useful method in preventing sheep abortions in Tabriz area.

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بررسی مولکولی(PCR) سقط جنین های ایجاد شده بوسیله گونه های مختلف کمپیلوباکتر در گوسفند داری های اطراف تبریز

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چکیده

کمپیلوباکتریوزیس یکی از بیماری های مهم عفونی انسان وحیوانات است که توسط گونه های بیماری زای کمپیلوباکترایجاد می-گردد. تعداد۱۳۲جنین سقط شده گوسفند بهمراه جفت های مربوطه از ماه مهر تااسفندماه سال۱۳۹۰از گوسفندداری های اطراف تبریز به کلینیک دامهای بزرگ دانشکده ارجاع داده شد. در آزمایشگاه ازمحتویات شیردان ،کبد،کلیه ،طحال ،ریه، مغز وجفت نمونه برداری شد وبعد از له شدن در ازت مایع تازمان استخراجDNA درفریزر۲۰ – درجه سانتیگراد نگهداری گردید. بعداز استخراج DNA (حضور ویا عدم حضورکمپیلوباکترهای مختلف (فتوس ، کولای وژژونای) توسط کیت تجاری Genomic DNA Extraction Kit, (AccuPrep Bioneer, S. Korea) بررسی شدند. براساس نتایج بدست آمده ۱۲ نمونه(۹/۰۹٪) نسبت به کمپیلوباکترفتوس فتوس و ۲ نمونه (۱/۵۱٪) نسبت به کمپیلوباکتر ژژونای واکنش مثبت نشان دادند. موردی از آلودگی جنین ها نسبت به کمپیلوباکتر کولای مشاهده نگردید.

واژگان کلیدی: کمپیلوباکتریوز ، سقط جنین، گوسفند، PCR